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(54) PROCESS FOR COMBATING FUNGI AND BACTERIA

(71) We, IMPERIAL CHEMICAL INDUSTRIES LIMITED, Imperial Chemical House, Millbank, London SW1P 3JF a British Company do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to the combating of fungi, bacteria and viruses which infest seeds, soil, plants and harvested produce.

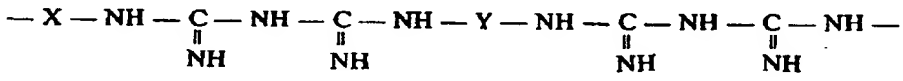
The efforts of mankind to grow useful crops, and to safely store the produce therefrom have long been hindered by the harmful and deleterious effects of fungi and bacteria.

In recent decades there has been a considerable increase in the use of chemicals to combat the numerous pests and diseases which adversely affect the efforts of those engaged in agriculture.

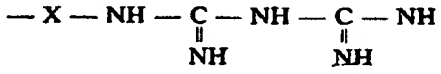
In recent years the efforts of researchers in the plant protection chemical field have been directed towards discovering chemical compounds having properties which minimise environmental hazards, and major advances have taken place in this direction. Thus there has been the discovery of the anti-fungal 2-amino-pyrimidines, known by the common names dimethirimol and ethirimol, which are relatively safe, non-toxic chemicals possessing a high level of anti-fungal activity.

It has now been discovered that a further class of relatively non-toxic chemicals possesses antifungal and antibacterial activity of such a kind that they may be used, surprisingly and remarkably, to combat certain fungi and bacteria which affect crops and harvested produce.

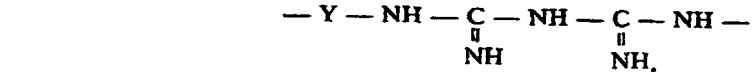
According to the present invention there is provided a method for combating fungi, bacteria and viruses which infest growing crops and the harvested produce obtained therefrom, which comprises treating the crops, or harvested produce, with a composition comprising, as an active ingredient, a polymeric biguanide or a salt thereof, which is in its free base form has a recurring polymer unit represented by the formula:—



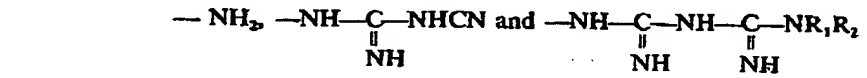
wherein X and Y, which may be the same or different, represent bridging groups $-(CH_2)_n-$ and $-(CH_2)_m-$ respectively, n and m having values from 3 to 12, or X and Y represent other bridging groups in which, taken together, the total number of carbon atoms directly interposed (as defined herein) between the pairs of nitrogen atoms linked by X and Y is from 10 to 16, and wherein the polymeric biguanide comprises a mixture of polymers in which the individual polymer chains are of different lengths, the number of individual polymer units:



and



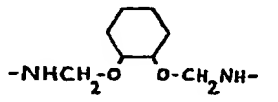
taken together in any p lymer chain being from 3 to 80, and wherein the groups terminating the polymer chains, which groups may be the same or different, are selected from



wherein R_1 is hydrogen or a substituted or unsubstituted aliphatic, cycloaliphatic, araliphatic or aromatic hydrocarbon radical containing from 1 to 18 carbon atoms and R_2 is a substituted or unsubstituted aliphatic, cycloaliphatic, araliphatic or aromatic hydrocarbon radical containing from 1 to 18 carbon atoms.

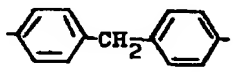
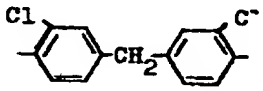
Specific polymeric compounds which have been prepared and found by tests to be bactericidally and fungicidally active are those wherein R_1 is hydrogen and R_2 is variously phenyl, 4-chlorophenyl, cyclohexyl, benzyl, 4-aminophenyl and cetyl. Other specific polymeric substances are listed on pages 5 and 6 hereinafter.

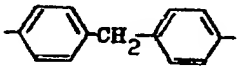
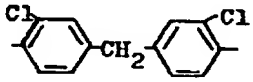
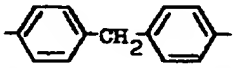
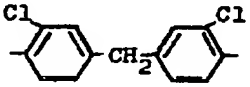
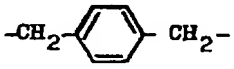
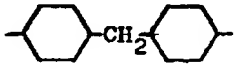
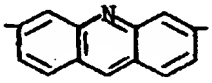
The bridging groups X and Y may consist of polymethylene chains, optionally interrupted by hereto atoms, for example, oxygen, sulphur or nitrogen. X and Y may also incorporate cyclic nuclei which may be saturated or unsaturated, in which case the number of carbon atoms directly, interposed between the pairs of nitrogen atoms linked X and Y is taken as including that segment of the cyclic group, or groups, which is the shortest, this defines the term "directly interposed", as used herein. Thus, the number of carbon atoms directly interposed between the nitrogen atoms in the group



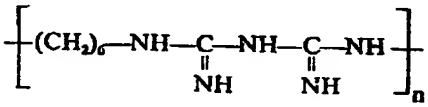
is 4 and not 8.

Examples of the polymeric biguanides which may be used are indicated below, each compound being defined by the divalent bridging radicals X and Y in the formula on page 3. In the case of these compounds the end groups, that is the groups terminating the polymer chains, are $-NH_2$ groups.

No.	X	Y
1	$-(CH_2)_2-$	$-(CH_2)_8-$
2	$-(CH_2)_2-$	$-(CH_2)_{12}-$
3	$-(CH_2)_2-$	
4	$-(CH_2)_2-$	
5	$-(CH_2)_3-$	$-(CH_2)_3-$
6	$-(CH_2)_3-$	$-(CH_2)_4-$
7	$-(CH_2)_3-$	$-(CH_2)_6-$

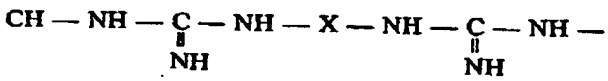
No.	X	Y
8	$-(CH_2)_3-$	$-(CH_2)_8-$
9	$-(CH_2)_3-$	$-(CH_2)_{12}-$
10	$-(CH_2)_3-$	
11	$-(CH_2)_3-$	
12	$-(CH_2)_6-$	$-(CH_2)_3-$
13	$-(CH_2)_6-$	$-(CH_2)_2-NH-(CH_2)_2-NH-(CH_2)_2-$
14	$-(CH_2)_6-$	$-(CH_2)_4-$
15	$-(CH_2)_6-$	$-(CH_2)_6-$
16	$-(CH_2)_6-$	$-(CH_2)_8-$
17	$-(CH_2)_6-$	$-(CH_2)_{12}-$
18	$-(CH_2)_6-$	
19	$-(CH_2)_6-$	
20	$-(CH_2)_6-$	
21	$-(CH_2)_6-$	
22	$-(CH_2)_7-$	$-(CH_2)_7-$
23	$-(CH_2)_6-$	$-(CH_2)_{10}-$
24	$-(CH_2)_{10}-$	$-(CH_2)_{10}-$
25	$-(CH_2)_6-$	

The preferred polymeric biguanide for use in the present invention is poly-(hexamethylene biguanide) which has the formula:—



wherein n has a value from 6 to 10, the average molecular weight of the polymer mixture being from 1100 to 1800. This material is preferably employed in the form of its hydrochloride salt, which is conveniently used as a 20% w/w aqueous solution (i.e.) 100 parts by weight of the solution contain 20 parts by weight of the active agent).

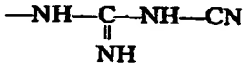
Polymeric biguanides may be prepared by the reaction of a bisdicyandiamide having the formula:



with a diamine $H_2N—Y—NH_2$, wherein X and Y have the meanings defined above; or by reaction between a diamine salt of dicyanimide having the formula

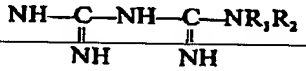
with a diamine $H_2N—Y—NH_2$ wherein X and Y have the meanings defined above. These methods of preparation are described in U.S. Patent Specifications Nos. 702,268 and 1,152,243 respectively, and any of the polymeric biguanides described therein may be used in the process according to the present invention.

The polymeric biguanides prepared according to either of the above described processes will have the polymer chains terminated either by an amino hydrochloride group or by an

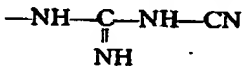


group, and the terminating group may be the same or different on each polymer chain.

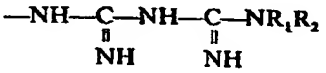
The polymeric biguanides which are partially or fully terminated by a



group (in the case of only one end of a polymer chain being terminated by the said group the other end will be terminated by an aminohydrochloride group or by an



group) are prepared by reacting 1 mole of dicyanimide or an equivalent amount of a metal salt thereof with approximately 0.5 mole of a diamine of the formula $H_2N—X—NH_2$ and reacting the product so obtained with a mixture of a diamine of the formula $H_2N—Y—NH_2$ and a monoamine of the formula R_1R_2NH, wherein X, Y, R_1 and R_2 have the meanings defined above. The preparation of these chain-stopped polymeric biguanides is fully described in U.K. Specification No. 1,167,249. The extent to which the polymeric biguanide is terminated by



groups depends upon the relative proportions of the diamine $H_2N—Y—NH_2$ and the monoamine R_1R_2NH which are used, and by varying this proportion products can be obtained in which the polymer chains are substantially entirely terminated

by the said groups or in which, on average, the polymer chains are only partially so terminated.

The polymeric biguanide salts which may be used in the invention process, and which are preferred therefor, include those derived from inorganic and organic acids.

Particularly preferred salts are those of the biguanide characterised by having the radicals X and Y in the general formula on page 3 constituted by hexamethylene groups, $-(CH_2)_6-$, and hereinafter referred to as polymeric hexamethylene diguanide. The terms "diguanide" and "biguanide" are synonymous.

The polymeric substances are freely soluble in water in the form of certain of their acid addition salts, such as their hydrochlorides, giving nearly neutral solutions which may be used in the invention process as such, or in conjunction with other substances, such as alkalis which give solutions of pH from 7 to about 12. There are indications that such solutions of high pH are more active fungicidally and bactericidally in the process of this invention. Much less soluble are metal salts, such as the copper salts, but these may also be used.

A number of different salts of polymeric hexamethylene diguanide have been found to possess anti-fungal and anti-bacterial properties and are therefore suitable for use in the process of the invention.

Examples of suitable salts include:

Salts of Inorganic Acids

Carbonate	Bromide
Sulphate	Metaphosphate
Phosphate	Hexametaphosphate
Nitrate	

Salts of Organic Acids

Formate	*p*-Toluene sulphonate
Benzoate	Adipate
Acetate	Citrate
Stearate	Succinate
Laurate	Caprylate
Dihydroacetate	Tartate
Phthalate	Glycollate
Sebacate	Malate
Behenate	Lactate
Gluconate	Trichloroacetate
Cinnamate	Malonate
Oleate	Myristate
	Maleate

Mixtures of these salts have also been prepared, as have partial salts of the free base, and these are also suitable for use in the process of the present invention.

The salts of polymeric hexamethylene diguanide may be prepared by any of the various well-known methods for making salts and to this end it is possible, for example, to commence either with the free-base itself, or with the highly water-soluble hydrochloride thereof. Thus the free base, or an aqueous solution of it, can be, if desired, added to the inorganic or organic acid, which may itself be in the form of an aqueous solution. Alternatively an aqueous solution of the hydrochloride salt of the polymeric biguanide may be added to, or have added to it, the sodium salt of the inorganic or organic acid, again if desired in the form of an aqueous solution thereof. The well-known techniques of ion-exchange may also be deployed to prepare these salts.

For a very considerable number of years the polymeric biguanides set forth in this specification have been used for disinfecting machinery.

There has, however, been no suggestion that these polymeric substances may be used in growing crops and harvested produce to combat the particular fungi and bacteria which infest them and which are of a different character from those previously combated.

The polymeric diguanides, particularly polymeric hexamethylene diguanide and salts thereof, are variously active against the following diseases:

A. Seed and Soil-Borne Fungal Diseases:

Latin Name for Disease	Examples of Host Crop	Ordinary or Common Nam of Disease
Fusarium culmorum	Wheat	Brown Foot Rot
Fusarium nivale	Rye	Brown Foot Rot
Septoria nodorum	Wheat	Glume Blotch
Fusarium oxysporum	Bananas	Panama Disease
Pyrenophora avenae	Oats	Leaf Blotch

B. Foliage-Borne Fungal Disease

Latin Name for Disease	Examples of Host Crop	Ordinary or Common Name of Disease
Podosphaera leucotricha	Apples and Pears	Powdery mildew
Piricularia oryzae	Rice	Rice blast
Erysiphe graminis	Wheat and Barley	Powdery mildew
Sphaerotheca mors-uvae	Blackcurrants	Powdery mildew
Erysiphe cichoracearum	Cantaloupes	Powdery mildew
Puccinia recondita	Wheat	Brown Rust
Uncinula necator	Vines	Powdery mildew
Colletotrichum lindemuthianum	Beans	Anthachose
Phytophthora infestans	Tomatoes	Late Blight
Plasmopara viticola	Vines	Downy Mildew
Ceratocystis ulmi	Elm Trees	Dutch Elm Disease
Botrytis cinerea	Tomatoes or Strawberries	Grey Mould
Mycosphaerella musicola	Bananas	Sigatoka leaf blight
Alternaria tenuis	Bananas	Leaf spot

C. Post-Harvest Fungal Diseases:—

Latin Name for Disease	Examples of Host Crop	Ordinary or Common Name of Diease
Fusarium roseum	Bananas	Crown rot complex
Botrytis tulipae	Bulbs	Fire
Thielavopsis basicola	Carrots	Black rot
~~*Nigrospora sphaerica*~~	~~Bananas~~	~~Squirter~~
Botrytis allii	Onion	Neck rot
Phomopsis citri	Citrus	Stem End Rot
Alternaria citri	Citrus	Stem End Rot
Penicillium expansum	Apples	Blue Mould
Penicillium digitatum	Citrus	Green Mould
Penicillium italicum	Citrus	Blue Mould
Gloeosporium musarum	Bananas	Anthracnose
Cladosporium musae	Bananas	Leaf Speckle
Botryodipiodia theobromae	Bananas	Blackend
Sclerotinia fructigena	Apples	Brown rot
Fusarium coeruleum	Potato	Dryrot
Ceratocystis paradoxa	Sugarcane, Pineapple	Pineapple Disease
Botrytis cinerea	Grapes	Grey Mould
Phoma exigua	Potato	Gangrene
Rhizopus stolonifer	Peaches	Rot
Phytophthora citrophthora	Citrus	Brown Rot
Diplodia natalensis.	Citrus	Stem End Rot
Sclerotinia fructicola	Peaches	Brown Rot

Latin Name for Disease	Examples of Host Crop	Ordinary or Common Name of Disease
Fusarium semitectum	Bananas	Crown Rot Complex
Geotrichum candidum	Citrus	Sour Rot
Verticillium theobromae	Bananas	Crown Rot Complex
Drechslera sacchari	Bananas	Crown Rot Complex
Curvularia senegalensis	Bananas	Crown Rot Complex
Rhizopus species	Raspberries	Rots

D. Bacterial Diseases:—

Latin Name for Disease	Examples of Host Crop	Ordinary or Common Name of Disease
Agrobacterium tumefaciens	Nursery Plants, Vegetables	Crown Gall
Corynebacterium michiganense	Tomato	Canker
Xanthomonas malvacearum	Cotton	Blackarm
Xanthomonas oryzae	Rice	Blight
Pseudomonas syringae	Beans, Stone Fruit	Dieback
Pseudomonas phaseolicola	Bean	Haloblight
Erwinia amylovora	Apple, Pear	Fireblight
Erwinia carotovora	Potato, Radish, Carrot	Soft Rot
Streptomyces scabies	Potato	Scab
Pseudomonas solanacearum	Tobacco	Granville Wilt
Leuconostoc mesenteroides	Sugar cane	Sour cane
Pseudomonas species	Cut Flowers, Celery Lettuce	

In addition to the foregoing diseases the polymeric biguanides may be used in combating the following diseases:—

Latin Disease Name	Common or Disease Name	Host Crop (Example)
Alternaria solani	Early Blight	Tomato/Potato
Ascochyta pisi	Pod spot	Peas
Aspergillus flavus	Mould	Nuts
Botrytis allii	Neck rot	Onion
Capnodium spp.	Sooty mould	Various plants
Cephaleuros parasitica	Red rust	Tea
Ceratostomella fimbriata	Rots	Sweet potato yam
Choanephora cucurbitarum	Wet rot	Cucurbits
Cladosporium fulvum	Leaf mould	Tomato
Cochliobolus miyabeanus	Brown spot	Rice
Marasmius perniciosus	Witches' broom	Cocoa
Marasmius spp.	Spear rot	Oil palm
	Decline	Pear
Mycoplasma spp.	Lethal Yellowing	Coconut
Penicillium spp., *Aspergillus spp.*, *Scopulariopsis* spp.	Post-harvest mould	Tobacco
Peronospora tabacina	Blue mould	Tobacco
Phoma betae	Blackleg	Sugar beet

Latin Disease Name	Common or Disease Nam	H st Crop (Examples)
Phytophthora palmivora	Blackpod	Cocoa
Plasmodiophora brassicae	Club root	Brassica
Pithomyces chartarum	Facial eczema of sheep	Grass
Pseudomonas pisi	Bacterial blight	Pea
Pseudomonas savastanoi	Knot	Olive
Pseudomonas solanacearum	Wilt, rot	Various crops
Rhynchosporium secalis	Leaf stripe	Cereals
Sclerotinia spp.	Drop	Lettuce
Septoria apii	Late blight	Celery
Spiroplasma citri	Stubborn	Citrus
Taphrina deformans	Leaf curl	Peach
Thielaviopsis basicola	Specific replant/ blackroot rot	Stone fruit/ tobacco
Tilletia caries	Bunt	Wheat
Xanthomonas campestris	Blackrot	Cabbage
Xanthomonas carotae	Blight	Carrot
Xanthomonas citri	Canker	Citrus
Xanthomonas phaseoli	Common blight	Bean
Xanthomonas vesicatoria	Bacterial leaf spot	Peppers/ Tomato

In carrying the invention process into practical effect the growing crops, plants, seeds, soil or harvested produce may be treated by any of the well-known and established procedures used in agriculture and crop protection. Thus, for example, the polymeric substances may be applied as solids, liquids, solutions, dispersions, emulsions and these may comprise, in addition to the active polymeric substance, any other adjuvant useful for formulation purposes, or any other biologically active substance, for example to increase the number of diseases combated.

Such solid or liquid substances and formulations may be applied, for example by any conventional technique, for example by dusting, or otherwise applying the solid substances and formulations to the surfaces of growing crops, harvested produce, plants, seeds or soil, or to any part, or combination of parts thereof, or, for example, applying liquids or solutions for example, by dipping, spraying, mist blowing or soaking techniques.

As used herein, the term harvested produce includes forage crops such as barley, oats, rice, sorghum and maize, and forage crops suitable for ensiling by treatment with the polymeric substances, exemplified by grass, maize, clover, lucerne, beans, peas, kale and sugar beets.

The invention process is therefore useful for treating plants, seeds, harvested fruits, harvested forage crops, vegetables, or cut flowers infested with, or liable to infestation with any of the aforementioned specific fungal or bacterial diseases.

The term "seeds" is intended to include propagative plant forms generally and therefore includes, for example, cut stems, corms, tubers and rhizomes.

The polymeric diguanides, or salts thereof, may be used as such but are preferably formulated into compositions for this purpose. Preferred compositions contain, as an active ingredient, polymeric hexamethylene diguanide.

In a further aspect, therefore, the invention provides a fungicidal or bactericidal composition for treating growing crops comprising, as an active ingredient, a polymeric diguanide as defined in any of the preceding paragraphs; together with a carrier substance therefor. The carrier may be a solid or liquid diluent. In the case of a liquid diluent being used, for example water, the composition may also contain a surface active (wetting) agent.

The compositions of the inventions may be in the form of dusting powders or granules wherein the active ingredient is mixed with a solid diluent or carrier.

Suitable diluents or carriers may be, for example kaolin, bentonite, kieselguhr, dolomite, calcium carbonate, talc, powdered magnesia, Fuller's earth, gypsum, Hewitt's earth, diatomaceous earth and China clay. Compositions for dressing seed, for example, may comprise an agent assisting the adhesion of the composition to the seed, for example a mineral oil.

The compositions may also be in the form of dispersible powders or grains comprising, in addition to the active ingredient, a wetting agent to facilitate the dispersion of the powder or grains in liquids. Such powders or grains may include fillers and suspending agents.

The compositions may also be in the form of liquid preparations to be used in the process of the invention for plants or harvested produce which are generally solutions, aqueous dispersions or emulsions containing the active ingredient in the presence of one or more wetting agents, dispersing agents, emulsifying agents or suspending agents.

Wetting agents, dispersing agents and emulsifying agents may be of the cationic, anionic or non-ionic type. Suitable agents of the cationic type include, for example quaternary ammonium compounds, for example, cetyltrimethyl ammonium bromide. Suitable agents of the anionic type include for example, soaps, salts of aliphatic monoesters or sulphuric acid, for example sodium lauryl sulphate, salts of sulphonated aromatic compounds, for example sodium dodecylbenzenesulphonate, sodium, calcium or ammonium lignosulphonate, butylnaphthalene sulphonate, and a mixture of the sodium salts of diisopropyl- and triisopropylnaphthalene sulphonic acids. Suitable agents of the non-ionic type include, for example, the condensation products of ethylene oxide with fatty alcohols such as oleyl alcohol or cetyl alcohol, or with alkyl phenols such as octylphenol, nonylphenol and octylcresol.

Other non-ionic agents are the partial esters derived from long chain fatty acids and hexitol anhydrides, the condensation products of the said partial esters with ethylene oxide, and the lecithins. Suitable suspending agents are, for example hydrophilic colloids, for examples polyvinylpyrrolidone and sodium carboxymethylcellulose, and the vegetable gums for example gum acacia and gum tragacanth.

The aqueous solutions, dispersions or emulsions may be prepared by dissolving the active ingredient in an organic solvent which may contain one or more wetting, dispersing or emulsifying agents. Suitable organic solvents are ethylene dichloride, isopropyl alcohol, propylene glycol, diacetone alcohol, toluene, kerosene, methylnaphthalene, xylenes and trichloroethylene.

The compositions to be used as sprays may also be in the form of aerosols wherein the formulation is held in a container under pressure in the presence of a propellant such as fluorotrichloromethane or dichlorodifluoromethane.

By the inclusion of suitable additives, for example for improving the distribution, adhesive power and resistance to rain on treated surfaces, the different compositions can be better adapted for the various uses for which they are intended.

The compositions may also be conveniently formulated by admixing them with fertilizers. A preferred composition of this type comprises granules of fertilizer material incorporating an invention compound. The fertilizer material may, for example comprise nitrogen, or phosphate — containing substances.

The compositions which are to be used in the form of aqueous dispersions or emulsions are generally supplied in the form of a concentrate containing a high proportion of the active ingredient, the said concentrate to be diluted with water before use.

The concentrates are often required to withstand storage for prolonged periods and after such storage, to be capable of dilution with water in order to form aqueous preparations which remain homogeneous for a sufficient time to enable them to be applied by conventional spray equipment.

The concentrates may conveniently contain from 4—85% and generally from 4—60% by weight of the active ingredient. A 20% aqueous solution is preferred. When diluted to form aqueous preparations, such preparations may contain varying amounts of the active ingredient depending upon the purpose of which they are to be used, but an aqueous preparation containing between 0.001% and 10% by weight of active ingredient may be used.

It is understood that the compositions of this invention may comprise; in addition to one or more polymeric substances according to the invention, one or

more other substanc s having biological activity, for example fungicidal, bactericidal, or insecticidal activity.

The substance polymeric hexamethyl ne diguanid hydrochloride is of low toxicity to mammals, the acute oral LD_{50} for rats being 100 mg/kg; no adverse effects were noted in animals given single doses of 500 mg/kg. Repeated application to the skins of rats of aqueous solutions is not irritant unless the concentration exceeds 5% (50,000 ppm ai). A 2.5% (25,000 ppm ai) solution in dimethyl formamide was not an allergic sensitiser and was non-irritant to the skin of guinea pigs. 0.1 ml of a 5% (50,000 ppm ai) aqueous solution caused no immediate or delayed irritation of rabbits' eyes.

In 90 days feeding tests no effect levels were established for rats of 625 ppm and for dogs of 2750 ppm in the diets.

The invention is illustrated but not limited by the following examples. In these Examples the compound polymeric hexamethylene diguanide hydrochloride may be referred to as P.H.D.H. as a convenient abbreviation.

EXAMPLE 1.

The activity of polymeric hexamethylene diguanide hydrochloride (P.H.D.H.) against a wide variety of plant bacterial and fungicidal diseases was investigated by *in vitro* tests as follows. 25 mg. of a 20% aqueous solution of the compound was added to 10 mg. of 10% aqueous acetone and 2 ml. of this was added to 18 ml. of nutrient agar (for the bacterial diseases) or 16 ml. of 2% malt agar (for the fungal diseases) to give a final concentration of 50 parts per million of the compound. Two ml. of a streptomycin preparation containing 100 units per millilitre was added to the malt agar to prevent bacterial contamination of the fungal tests.

The agar preparations were dried overnight in petri dishes and inoculated the following morning with the bacterial or fungal diseases using a multipoint inoculator. The antibacterial activity was assessed after 5 days and the antifungal activity after 6 days.

The results of the tests are set out below in the Tables. The results are graded as set out below. The names of the disease organisms are indicated in the first Table.

TABLE

Bacterial Disease	Code	Fungal Disease	Code
Agrobacterium tumefaciens	B1	*Nigrospora sphaerica*	F1
Corynebacterium michiganese	B2	*Phytophthora citrophthora*	F2
Xanthomonas malvacearum	B3	*Alternaria citri*	F3
Erwinia carotovora	B4	*Diplodia Natalensis*	F4
Xanthomonas oryzae	B5	*Phomopsis citri*	F5
Pseudomonas syringae	B6	*Ceratocystis paradoxa*	F6
Streptomyces scabies	B7	*Gloeosporium musarum*	F7
Pseudomonas phaseolicola	B8	*Penicillium digitatum*	F8
		Phoma exigua	F9
Erwinia amylovora	B9	*Botrytis tulipae*	F10
		Botryodiplodia theobromae	F11
		Fusarium coeruleum	F12

In the Tables below the significance of the gradings is as follows:—

0 = no control

1 = slight control

2 = moderate control

3 = complete control

BACTERIAL DISEASES

Disease Code

B1	B2	B3	B4	B5	B6	B7	B8	B9
3	3	3	3	3	3	3	3	3

FUNGAL DISEASES

Disease Code

F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
3	3	3	3	3	3	3	3	3	3	3	3

EXAMPLE 2.

This example illustrates the *in vivo* use of polymeric hexamethylene diguanide hydrochloride, and other salts, to combat post-harvest fungal infections affecting oranges and bananas. Various compositions containing polymeric hexamethylene diguanide salts were used, and compared with the compound known by the British Standards Institution common name benomyl (1-*n*-butyl carbamoyl-2-benzimidazole carbamic acid methyl ester). The test conducted was an eradicant and protectant dip-test against the diseases *Penicillium digitatum* (green mould of citrus) and *Gloeosporium musarum* (anthracnose of bananas). The procedure adopted was as follows:

Four discs 10 mm in diameter, or oranges and banana peel, are dipped in aqueous suspensions containing 100, 500 and 1000 parts per million (p.p.m.) of test chemical ether (in eradicant tests) 1 day after inoculation with either *Penicillium digitatum* or *Gloeosporium musarum* spore suspension 10^6 cells/ml) or (in protectant pests) 3 hours before. The discs are randomly placed in five separate plastic "Replidishes" in which the relative humidity is kept high with moist filter paper for 1 week. The discs are scored for disease on a 0—4 scale. If all discs were completely healthy the treatment scored a 4; if only three dishes were healthy it scored a 3; if only two discs were healthy it scored a 2; if only one a 1, or if none was healthy the score was 0. Both eradicant and protectant treatments were assessed together. The results are set out in the Table below.

"Replidish" is a Trade Name for a 10 x 10 centimeter petri dish sub-divided into 35 cube compartments sealed off from each other by a vertical plastic partition.

HYDROCHLORIDE SALT

P.H.D.H. Rate in p.p.m.	Treatment	Disease	Score
1000	Eradicant	*Gloeosporium musarum*	4
1000	Protectant	*Gloeosporium musarum*	4
500	Eradicant	*Gloeosporium musarum*	4
500	Protectant	*Gloeosporium musarum*	4
100	Eradicant	*Gloeosporium musarum*	4
100	Protectant	*Gloeosporium musarum*	4
1000	Eradicant	*Penicillium digitatum*	4
1000	Protectant	*Penicillium digitatum*	4
500	Eradicant	*Penicillium digitatum*	4
500	Protectant	*Penicillium digitatum*	4
100	Eradicant	*Penicillium digitatum*	4
100	Protectant	*Penicillium digitatum*	4

OTHER SALTS

Fungal Disease	Salt (at 100 ppm rate)		
	Acetate	Gluconate	Sulphate
Penicillium digitatum			
Eradicant	3	3	4
Protectant	4	4	3-4
Gloeosporium musarum			
Eradicant	2-3	3-4	4
Protectant	4	4	4

EXAMPLE 3.

In a further test carried out in Spain an aqueous solution comprising 1000 and 2000 p.p.m. of polymeric hexamethylene diguanide hydrochloride was compared with benomyl. Whole oranges were dipped in the test chemicals or in water. These fruits were then waxed with a standard citrus wax, and stored. The fruits were assessed on two occasions for the percentage number of them which were infected with *Penicillium digitatum* and *Alternaria citri;* the total number of rotted fruits was counted. The results are set out in Tables below.

PERCENTAGE OF ORANGES ROTTED PER BOX (BOTH DISEASES)

	1st Assessment (Penicillium digitatum only)	2nd Assessment
Polymeric hexamethylene diguanide hydrochloride 1000 ppm	0.6	7.3
Polymeric hexamethylene diguanide hydrochloride 1000 ppm+ Agral 90 0.3% *	0.8	0.6
Polymeric hexamethylene diguanide hydrochloride 2000 ppm	0.6	4.1
Polymeric hexamethyelene diguanide hydrochloride 2000 ppm+	0.0	0.6
Benomyl 1000 ppm	0.2	4.3
Untreated	8.2	19.6

* Agral 90 is a wetting agent comprising 90% Lissapol NX and 10% industrial methanol. Lissapol NX is a condensate of 1 mole of nonyl phenol with 9 moles of ethylene oxide. "Agral" is a Registered Trade Mark.

\+ At first assessment only *P. digitatum* was present.

PERCENTAGE OF *PENICILLIUM DIGITATUM* INFECTED ORANGES PER BOX

	1st Assessment	2nd Assessment
Polymeric hexamethylene diguanide hydrochloride 1000 ppm	0.6	6.5
Polymeric hexamethylene diguanide hydrochloride 1000 ppm + Agral 90 0.03%	0.8	0.6
Polymeric hexamethylene diguanide hydrochloride 2000 ppm	0.6	4.1
Polymeric hexamethylene diguanide hydrochloride 2000 ppm + Agral 90 0.3%	0.0	0.6
Benomyl 1000 ppm	0.2	1.1
Untreated	8.2	17.8

PERCENTAGE NUMBER OF ALTERNARIA CITRI INFECTED ORANGES PER BOX

	2nd Assessment*
Polymeric hexamethylene diguanide hydrochloride 1000 ppm	0.1
Polymeric hexamethylene diguanide hydrochloride 1000 ppm + Agral 90 0.03%	0.0
Polymeric hexamethylene diguanide hydrochloride 2000 ppm	0.0
Polymeric hexamethylene diguanide hydrochloride 2000 ppm + Agral 90 0.03%	0.0
Benomyl 1000 ppm	2.24
Untreated	0.56

* No *A. citri* detected at first assessment

EXAMPLE 4.

This example illustrates the use of polymeric hexamethylene diguanide hydrochloride to combat the post harvest fungal rot of potatoes caused by the organism *Fusarium coeruleum*. In this test 3 replicates of eight freshly cut quarters of potato tubers (cultivar Record) were treated by dusting with a standard fungicide, TCNB dust, used to protect tubers against *Fusarium coeruleum* or were dipped in an aqueous solution containing 150 ppm of the test compound, or left untreated. When dry these tubers were sprayed with a suspension of 4×10^3 spores per millilitre of a culture of *Fusarium coeruleum*, and placed in an open polythene bag and stored at 150°C. The number of rotted tuber quarters was assessed after 5 and 7 days. The results are shown in the Table below.

Treatment	Number of tuber quarters rotted	
	After 5 days	After 7 days
Polymeric hexamethylene diguanide hydrochloride	4	4
*Formulated hydrochloride	0	4
** TCNB	18	20
Untreated control	23	24

*This comprised the chemical plus surface active agents.

** TCNB is tetrachloronitrobenzene.

EXAMPLE 5.

This example illustrates the activity of polymeric hexamethylene diguanide hydrochloride against the disease *Streptomyces scabies* (potato scab).

The test procedure was as follows:— Soil was taken from the top 10 cm. of an infested field, thoroughly air-dried, sieved, mixed and stored until needed. Small shoots, obtained from tubers of scab-susceptible potatoes (Cultivar Red Craigs

Royal McIntosh and Eveling, 1965), wer planted in potting compost in seed boxes for 1—2 weeks, so that th shoots grew to a height of 7 centimetres or more.

Inoculum, for boosting th natural infectivity of the field soil, was made by drying the contents of liquid shake-cultures (Vruggink and Maat, 1968) on to field soil, using about 150 millilitres per kilogram of soil.

Booster inoculum (about 50 g/kg) and test chemical (250 milligrams of a 20% w/v aqueous solution) were thoroughly mixed with field soil. Pots (12 cm. diam.) were filled with three layers of soil of equal depth. The bottom layer was potting compost and the middle layer treated field soil; a circle of 'Terylene' net; (0.5 mm.mesh; "Terylene" is a Registered Trade Mark), large enough to reach up the sides of the pot to the soil surface, separated the middle layer from the top layer, which was also of treated field soil. One rooted shoot was transplanted into the top layer of each pot.

The pots were suitably randomized in a glasshouse or growth room, with minimum temperature of 20°C (day) and 15°C (night). They were watered freely for the first 10 days, but after that were placed on a sand bed without overhead watering. During very hot weather extra water was given as necessary.

The tubers, harvested 8—10 weeks after potting, were weighed and graded for scab infection (Large and Honey, 1955; Lapwood and Dyson, 1966) to give yield and mean 'scab index' per pot. Results from five pots per treatment, were assessed giving mean scab indices.

The test chemical gave control of the disease equivalent to PCNB applied at the same rate of 50 ppm (a known standard treatment).

PCNB is pentachloronitrobenzene.

EXAMPLE 6.

This example illustrates further the use of PHDH against soft rot of potatoes. Potato discs 10 mm. in diameter and about 1 mm. thick were cut from tubers (variety Red Craig's Royal). Four discs were dipped in PHDH aqueous solutions and in water alone as a control. Tests were also conducted, for a comparison with sodium hypochlorite and streptomycin at 500 ppm. The discs were randomly placed in four plastic "Replidishes", one for each replicate disc, and on to each disc was pipetted 0.1 ml. of a suspension of *Erwinia carotovora* containing 10^9 cells per ml. The dishes were incubated for 24 hours at 25°C maintaining humidity with damp tissue paper placed in the lids. The potatoe discs were scored for the presence or absence of soft rot, and the discs not rotted were totalled to give scores ranging from 4 (for all healthy) to 0 (for all rotted). The results are shown below.

Chemical	PPM Rate	Soft Rot Grading
PHDH	500	4
PHDH	200	3
PHDH	100	2
PHDH	50	1
PHDH	20	0
Water Control	—	0
Sodium hypochlorite	500	0
Streptomycin	500	2

In a further experiment various other salts of polymeric hexamethylene diguanide were tested in the same way and the results are tabulated below.

Salt (at 100 ppm)	Disease Control Rating
Acetate	4
Sulphate	4
Gluconate	4

EXAMPLE 7.

This Example further illustrates the activity of polymeric hexamethylene diguanide hydrochloride against *Erwinia carotovora.*

Two glasshouse experiments were carried out, one using cut seed potato pieces and the other whole seed potatoes. These were dipped in a solution containing 500 ppm of the test compound for half an hour and sprayed when dry

with a suspension of a cultur of *Erwinia carotovora* (10^9 cells per millilitre) and then planted. The emergence of the potato shoots is given below in the Table. It is clear that the test compound improves potato emergence, but that the addition of Cetrimide is disadvantageous.

TABLE

Compound	Rate in ppm of Test Chemical	TYPE OF SEED POTATO	
		Cultivar 'Arran Pilot' percentage emergent Shoots (Seed Pieces)	Cultivar 'Ulster Chieftan' Number of shoots which emerged (whole seed)
Polymeric hexamethyl diguanide hydrochloride	500	75	16
Polymeric hexamethyl diguanide hydrochloride + cetrimide	500+500	50	0
Sodium hypochlorite	500	0	-
R.E. 49*	0.2%	0	1
Agrimycin**	500	100	9
Control untreated	-	0	8

* R.E.49 is a standard composition containing dichlorophen which is 5,5'-dichloro-2,2'-dihydroxyphenylmethane.
** Agrimycin is a 10:1 mixture of streptomycin and tetracycline.

EXAMPLE 8.

This Example further illustrates the activity of polymeric hexamethylene diguanide hydrochloride against *Erwinia carotovora.*

Seed potatoes (Cultivar Red Craigs Royal) were dipped in a solution containing 500 ppm of the test chemical alone and separately in a solution containing 500 ppm of the test chemical and 500 ppm of cetrimide. Three days later they were dipped in a 10^9 cells/ml suspension of *Erwinia carotovora.* 4 replicates of 25 tubers were planted in ridges together with untreated controls which had been similarly inoculated, and also some uninoculated controls. 47 days later the number of plants which had emerged was assessed. The results are shown in the Table below. It is clear that the test chemical improved the emergence of the potato plants by combating the disease.

Test Compound	Rate in p.p.m. of test chemical	Number of Plants which emerged	Percentage emergence
Polymeric hexamethylene diguanide hydrochloride	500	18.2	73.5
Polymeric hexamethylene diguanide hydrochloride + cetrimide	500	10.7	42.1
Control (inoculated)	-	6.7	26.8
Control (uninoculated	-	10.7	43.0

EXAMPLE 9.

This Exampl also illustrates the us of PHDH against potato rots.

Potatoes, variety Red Craigs Royal, were dipped in PHDH aqu ous solutions at 5,000, 1,000 200 and 100 ppm, and also in water alon as a control. 100 tubers were dipped in each solution and in th water alone. These were split up into 5 replicates of 20 tubers each and stored in sealed polythene bags with holes punched in them at 22°C.

They were assessed after 7, 12, 19 and 30 days for storage diseases due to bacterial soft rot. The results are shown in the table below as gradings on a scale from 0.00 (completely rot-free) to 4.00 (completely rotted).

Treatment	7 days	12 days	19 days	30 days
PHDH 5000 ppm	0.00	0.00	0.00	0.00
1000 ppm	0.00	0.05	0.10	0.12
200 ppm	0.05	0.17	0.20	0.28
100 ppm	0.03	0.08	0.08	0.10
Water control	0.99	0.78	0.72	1.04

PHDH at rates from 5000 to 100 ppm is therefore apparently effective in controlling bacterial soft rot of potatoes *(Erwinia carotovora)*.

EXAMPLE 10.

This Example illustrates the activity of PHDH against bacterial soft rot *(Erwinia carotovora)* of brussel sprouts.

Four replicates, each consisting of 2 lb. weight of brussel sprouts, were used. The vegetables were dipped in aqueous solutions containing 500 and 1000 ppm of PHDH, and in water alone as an untreated control.

Results are given in the table below:—

Chemical	Rate (ppm)	Percentage amount of disease
PHDH	1000	35.5
PHDH	500	15.0
Control	-	62.9

Significant control of the bacterial soft rot was given. In this test supplies of healthy brussel sprouts were obtained together with other samples infected bacterial soft rot. The trial samples were contaminated with the infected product by stirring the two together in a drum full of water. The sprouts were then dipped in the PHDH solutions, and in water alone, for one minute. They were then placed in sealed plastic bags and incubated at room temperature.

EXAMPLE 11.

This Example illustrates the use of PHDH as a prepack dip against postharvest rots of tomatoes (*Penicillium* species).

Glasshouse grown tomatoes were freshly harvested and dipped in aqueous solutions of PHDH containing respectively, 1000, and 125 ppm. Benomyl (50% Dispersible Powder) at 200 ppm was used as a standard. The tomatoes were left to dry and were packed in small polythene bags with holes punched into them. Eight fruits were placed in each bag and there were 5 replicates per treatment. The bags were left open and placed in a 25°C constant temperature room. They were examined frequently. No rotting began until 2 weeks after assessment. Fungal and

bacterial rots were prevalent especially *Penicillium* species. Assessments were made 2 and 3 weeks after treatment and the percentage (%) number of healthy fruits was as shown below:—

Treatment	Percentage number of healthy fruits after 2 weeks.	% after 3 weeks
1000 ppm PHDH	99.5	77.6
125 ppm PHDH	97.9	81.4
BENOMYL - 200 ppm	95.7	67.8
Water Control	72.5	23.9

PHDH at 125 ppm therefore, gives better rot control than Benomyl. At 1000 ppm rot control is even better.

EXAMPLE 12.

This Example illustrates the use of PHDH as a pre-pack dip against post harvest rots of carrots. Carrots, variety Chantenay, were dipped in PHDH aqueous solution containing 400, 200,100 and 40 ppm respectively of PHDH both with and without the presence of 300 ppm of the surface active agent at 300 ppm. Sodium hypochlorite at 40 ppm and water were used as standard and control dips, respectively. The carrots were packed wet in polythene bags with holes and 5 replicate bags each containing 5 carrots were used for each treatment. The bags were then closed. The carrots were stored in boxes at 22°C. They were assessed both 7 and 11 days later for rots and the results are shown in the table below. Both bacterial and fungal rots, especially *Thielaviopsis basicola* occurred. The diseases were assessed and graded on a scale 4.00 (completely rotted) to 0.00 (completely free of disease).

Treatment	Soft Rot		Thielaviopsis basicola	
	7 days	11 days	7 days	11 days
PHDH 400 ppm	-	-	0.00	0.00
" 200 ppm	0.60	1.12	0.16	0.24
" 100 ppm	0.80	1.32	0.40	1.08
" 40 ppm	0.52	1.64	1.04	1.56
PHDH 400 + Agral 90 300 ppm	2.20	2.35	0.00	0.10
" 200 + " "	2.00	2.50	0.00	0.45
" 100 + " "	0.36	1.04	1.04	2.80
" 40 + " "	0.72	2.04	1.36	1.76
Sodium hypochlorite 40 ppm	0.68	4.00	3.92	4.00
Water control	0.32	4.00	4.00	4.00

These results demonstrate that black rot of carrots (the fungal disease *Thielaviopsis basicola*) is controlled by PHDH at various concentrations and that control of soft rots over the longer period (11 days) is also obtained.

EXAMPLE 13.

This Example illustrates the use of PHDH against postharvest rots of Radishes (variety Short top forcing — Tozer). Radishes were dipped in aqueous solutions containing 400, 200, 100 and 40 ppm of PHDH with and without "Agral" 90 at 300 ppm. They were placed wet in polystyrene trays, 25 radishes in each tray, and covered with self-sealing "Cellophane" wrap ("Cellophane" is a Registered Trade Mark). There were four replicate tray- per treatment. These were stored at 22°C and observed for storage diseases. The rots appeared very slowly, a few occurring after a week. They were assessed 16 days after dipping. Bacterial soft rot (*Erwinia carotovora*) was the main disease present. The results are shown below:—

Treatment	Percentage No. of Soft-rotted Radishes.
PHDH 400 ppm	12.0
200 ppm	3.6
100 ppm	7.4
40 ppm	9.5
PHDH 400 ppm + Agral 90 300 ppm	5.7
200 ppm "	4.4
100 ppm "	9.6
40 ppm "	23.1
Sodium hypochlorite - 40 ppm	81.6
Water control	99.5

The above results demonstrate the capacity of aqueous solutions of PHDH, with and without the presence of added surface agent, to hinder rotting in stored radishes. Considerably better control than that given by sodium hypochlorite is achieved.

EXAMPLE 14.

The activity of polymeric hexamethylene diguanide hydrochloride (PHDH) against fungal and bacterial organisms causing rots in produce pre-packed for sale in polythene or similar containers was investigated by *in vivo* tests as described below:—

Commercially-prepared vegetables (whole, shredded, pre-washed or otherwise processed) were dipped in aqueous solutions containing various concentrations of polymeric hexamethylene diguanide hydrochloride. An untreated control treatment in which the produce was dipped in water only was included in all experiments. The water used was water normally used for washing the produce commercially. In all experiments 50 litres of each test solution was prepared in large rigid polythene containers. The prepared produce was placed into polythene mesh nets and immersed in the solutions for two minutes, after which time it was taken out and put out to dry in trays for a few minutes. The produce from each treatment was split into 4 replicates and packed into polythene bags or similar containers. These were arranged in randomised block designs with 10 replications. The produce was stored at 22°C to encourage rots to develop. The amount of produce per pack was normally the same as that prepared for sale commercially.

Assessment for rots was carried out at intervals after dipping. The produce was assessed for Tests D and E for its general appearance on a 0—4 or 0—5 basis where 0 represents a good appearance and 4 or 5 represents a badly damaged appearance. In the assessments for Tests A—C the level at which the produce was considered unsaleable was recorded in order that the percentage of unsaleable produce could be calculated.

The results of the tests on a range of produce are set out below in Tables A—E.

TABLE A — Carrots (assessed 8 days after dipping).

	Percentage amount of unsaleable Carrots
PHDH — 50 ppm	6.1
„ 200 ppm	7.5
Untreated control	14.2

The carrots were partially scrubbed before dipping. After dipping they were stored in open, perforated polythene bags.

TABLE B — Celery (assessed 8 days after dipping)

	Percentage amount of unsaleable celery
PHDH — 50 ppm	20.6
„ 100 ppm	20.6
Untreated control	97.6

The celery was trimmed, washed and its outer leaves removed, before dipping. It was then stored in open perforated polythene bags.

TABLE C — Leeks

	Percentage amount of unsaleable leeks
PHDH — 50 ppm	1.5
„ 100 ppm	1.5
„ 200 ppm	0.0
Untreated control	20.1

The leeks were trimmed and the outer leaves removed before dipping. They were then stored in open perforated polythene bags.

TABLE D — Lettuce
General appearance assessment.

	Mean grading scored
PHDH — 50 ppm	1.70
Untreated control	2.80
	(4 days post dipping)

The lettuces received no pre-treatment wash. After dipping the lettuces were stored in open, perforated polythene bags.

TABLE E — Cabbage
General appearance assessment.

	Mean grading scored
PHDH — 50 ppm	0.7
„ 100 ppm	0.2
„ 200 ppm	0.2
Untreated control	1.5

The cabbage was shredded before treatment and stored after treatment in a polystyrene tray sealed with polythene film.

The rots causing damage were predominently bacterial organisms fro, the genera *Erwinia* and *Pseudomonas*. Aqueous solutions of PHDH were clearly efficacious in combating fungal/bacterial rotting of the vegetables.

EXAMPLE 15.

This Example illustrates the use of PHDH as a postharvest dip for apples to combat storage rots.

Two hundred apples, variety Cox, were dipped in aqueous solutions containing 1000 and 500 ppm of PHDH alone, and at 1000 ppm together with "Agral" 90 wetter at 300 ppm. They were then placed in boxes, four replicates being deployed, of which each contained 50 apples, and then stored at 22°C. They were

assessed for storag rots, mainly blue mould, *Penicillium expansum*, and brown rot, *Sclerotinia fructigena* after storage periods of 30 and 38 days. The results are shown in the table below:—

Treatment	Percentage amount of healthy fruit	
	30 days	38 days
PHDH at 1000 ppm	78.8	63.0
PHDH at 500 ppm	81.8	64.8
PHDH 1000 ppm + "Agral" 90 at 300 ppm	85.3	75.8
Water Control (untreated)	76.9	58.2

From these results it appears that PHDH is effective in controlling postharvest storage rots of apples, especially when used in conjunction with a surface active agent.

EXAMPLE 16.

This Example illustrates the treatment of raspberries, variety Malling Jewel, with PHDH to preserve them.

Raspberries were sprayed to wetness before picking with a variety of compositions as follows:—

Treatment No.	Composition
1	Aqueous solution containing 2000 ppm PHDH
2	" " " 1000 ppm PHDH
3	" " " 2000 ppm PHDH
4	" dispersion " 2000 ppm of copper salt of PHDH
5	Benomyl 500 ppm
6	Untreated control

The same day the raspberries were innoculated with spores of *Botrytis Cinerea* using a "Killaspray" (Trade Name) hand sprayer and an inoculum containing 200,000 spores per millilitre suspended in a 1% aqueous sucrose solution. The inoculation was effected by spraying intermittently along the rows of raspberry canes at alternating untreated areas and treated areas. A week later a second inoculation was carried out in similar fashion. Eight days later the ripe raspberries were harvested and the same day the remainder, (mostly green and pale) were given a second treatment with the chemical compositions; then a third inoculation was carried out as before. Three days later the harvested fruit were assessed for infection, having been sorted into petri dishes after picking, and held at 65°F and 100% relative humidity for 48 hours. Thereafter they were removed from the humidity cabinet and allowed to stand for 24 hours for disease development. The fruit remaining of the canes which had ripened in the six days after the last inoculation were then harvested, treated in a similar fashion to those previously harvested (but kept in the humidity cabinet for 68 hours) and then assessed for

development of infection. Assessment was a visual inspection f individual fruits.

The infection observed included not only *Botrytis cinerea*, but also extraneously occurring rots of *Penicillium* and *Rhizopus* species.

	Treatment No.					
	1	2	3	4	5	6 Untreated control
First picking	36.3	39.5	47	21.2	29	10
Second picking	30	28	33	29	21	22

The figures given in the above table are the percentage number of uninfected raspberries. (Average of 5 separate lots — approximately 250 fruits).

EXAMPLE 17.

This Example illustrates the use of a polymeric hexamethylene diguanide (hydrochloride (PHDH) as a dip treatment to combat postharvest rots of peaches.

Peaches were dipped in polymeric hexamethylene diguanide hydrochloride solutions at 1000 ppm and 2000 ppm. Benomyl at 1000 ppm, dicloran 1875 ppm and a mixture of 500 ppm benomyl and 1875 ppm dicloran were used as standard treatments and water dips were used as the untreated control. After dipping, the fruit was stored. Subsequently it was examined for infected fruit and the results of this assessment are given in the table below. The rots were mainly due to *Rhizopus* sp but *Sclerotinia fructicola* was also present.

No.	Treatment	Percentage Amount of diseased fruit after 2 days storage.
1	Polymeric hexamethylene diguanide hydrochloride 1000 ppm	28
2.	" " 2000 ppm	21
3.	Benomyl 1000 ppm	42

No.	Tr atment	Percentag Amount of diseased fruit after 2 days storage.
4.	Dicloran 1875 ppm	18
5	benomyl 500 ppm + dicloran 1875 ppm	19
6	water	56

In a similar test polymeric diguanide hydrochloride was effective at even lower rates against *Rhizopus nigricans*. A *Penicillium* sp was also present.

No.	Treatment	Percentage Amount of diseased fruit after 3 days storage
1.	Polymeric hexamethylene diguamide hydrochloride 250 ppm	30
2.	" " 500 ppm	14
3.	Maneb 1000 ppm	48
4.	Water	38

In this trial Maneb, but not polymeric hexamethyiene diguanide hydrochloride was highly phytotoxic.

These results show that polymeric hexamethylene diguanide hyrochloride is more effective as a post-harvest dip than benomyl alone or Maneb alone against *Rhizopus* and other rots of peaches.

EXAMPLE 18.

This Example illustrates the activity of polymeric hexamethylene diguanide hydrochloride against disease of sugar cane.

Six slices of 1 millimetre thickness were taken from sugar cane setts (Cultivar Natal-Coimbatore 376) and dipped for 10 minutes in the test chemical, benomyl or "Aretan 6" ("Aretan" is a Registered Trade Mark) (6% ethoxyethyl mercurichloride). When dry the sett slices were placed in a petri dish with 0.2 ml from a 340,000 spore/ml suspension of *Ceratocystis paradoxa* spores, the causal agent of pineapple disease of sugar cane. The petri dishes were then kept for 7 days and then assessed for mycelial growth. The Table below illustrates that the test chemical gives equivalent control of the disease to benomyl and "Aretan 6".

Chemical Compound used.	Rate in ppm of Active Chemical.	Number of slices with mycelial growth
Benomyl	7000	0
"Aretan 6"	900	0
Polymeric hexamethylene diguanide hydrochloride (4% aqueous solution)	3500	0
Polymeric hexamethylene diguanide hydrochloride(4% aqueous solution)	7000	0
Polymeric hexamethylene diguanide hydrochloride (20% aqueous solution)	7000	0
Untreated		6

EXAMPLE 19.

This Example further illustrates the usefulness of polymeric hexamethylene diguanide hydrochloride for the post harvest preservation of fruit. In this test 6 green unripe hands of bananas were each dipped in a 20% aqueous solution of the diguanide, the diguanide plus gibberellic acid, benomyl alone, gibberellic acid alone, or benomyl plus gibberellic acid for 5 minutes. The fruit was then stored at 20°C for 14 days until some started to ripen, and assessed for ripening, as summarised in the Table below:—

Treatment	Number of Green Hands after 14 days
Benomyl 250 ppm	1
Benomyl 250 ppm + Gibberellic Acid 100 ppm	1
Gibberellic Acid 100 ppm	3
Polymeric hexamethylene diguanide hydrochloride 1000 ppm	1
Polymeric hexamethylene diguanide hydrochloride 1000 ppm + Gibberellic Acid 100 ppm	5
Untreated	0

This example shows the surprising synergism for preventing ripening of fruit between the test chemical and gibberellic acid and thus its usefulness in perserving and lengthening the storage life of harvested fruit.

EXAMPLE 20.

Polymeric hexamethylene diguanide as the free base, certain of its salts, and most of the polymeric biguanides numbers 1 to 25 on pages 5 and 6 were tested against a variety of foliar fungal disease of plants. The technique employed is to spray the foliage of the undiseased plants with a solution of the test compound and also to drench the soil in which the plants are growing with another solution of the test compound.

All solutions for spraying contained 0.1% of the test compound. All the soil drench solutions also contained 0.1% of the test compound.

The plants were then infected with the disease it was desired to control and after a period of days, depending upon the particular disease, the extent of the diseases was visually assessed. The results are given below, in the form of a grading as follows:—

Grading	Percentage Amount of Disease
0	61 to 100
1	26 to 60
2	6 to 25
3	0 to 5

In the first Table below, the disease is given in the first column, whilst in the second column is given the time which elapsed between infecting the plants and assessing the amount of disease. The third column assigns to each disease a code letter, these code letters being used in the Second Table to identify the diseases.

TABLE

Disease and Plant	Time interval (days)	Disease Code letter (Table No. 2)
1) *Puccinia recondita* (wheat)	10	A
2) *Phytophthora infestans* (tomato)	3	B
3) *Plasmopara viticola* (vine)	7	C
4) *Uncinula necator* (vine)	10	D
5) *Piricularia oryzae* (rice)	7	E
6) *Podosphaera leucotricha* (apple)	10	F
7) *Botrytis cinerea* (broad bean)	3	G

TABLE

Compound	Disease Code Letter						
	A	B	C	D	E	F	G
Polymeric hexamethylene diguanide (free base)	1	3	2	2-3	2-3	0	3
Polymeric hexamethylene diguanide sulphate salt	2-3	2	0-2	0	0	0	3
Polymeric hexamethylene diguanide hydrochloride	1	0-1	1-3	0-1	3	0	3
Polymeric hexamethylene diguanide carbonate	1	0-1	2-3	0-3	1	0	3
Polymeric hexamethylene diguanide digluconate	0-1	0	2-3	1-2	2-3	0	3
Polymeric hexamethylene diguanide benzoate	1	3	1-3	2-3	3	3	3
Polymeric hexamethylene diguanide phthalate	2-3	2-3	3	2	2-3	0	3
Polymeric hexamethylene diguanide acetate	3	3	0-1	3	2	0	3
1	2	0-1	3	0-2	0	0	1-3
2	1	0	1	0	0	0	1-3
3	0	0-1	3	0	0	0	3
4	0	0-1	3	0-3	0	0-3	2-3
5	0	0	1	0-3	0	0-3	2-3
6	0	1	3	1-2	0	0	3
7	0	1	1	0	3	0	3
8	2	0	0	3	0	0	3

TABLE (continued)

Compound	Disease Code Letter						
	A	B	C	D	E	F	G
9	0	2	1	0-1	0	0	2-3
10	0	0	0	0	0	0	2-3
11	0	0	0	0-2	0	0	2-3
12	0	0	0	0	0	0	3
13	0	2	0	0	0	2	0
14	0	0	0	0	0	0	1-3
15	0	3	0	3	0	0	2
16	0	2-3	3	2-3	1-3	0	3
17	0	0	0	0-1	0	0	0
18	1	0-2	1	0	0	0	3
19	0	0	0	0-1	0	0	3
21	0	0	1	0	0-3	0	1-3
22	0	0	1	1	0	0	3
25	0	0	0	0	0-3	0	0

EXAMPLE 21.

This Example illustrates th use of PHDH to c mbat the fungal disease *Puccinia recondita* (wheat rust).

Wheat plants (variety Jufy 1) one week old, grown in 3" diameter pots (about 20 plants per pot) under controlled environmental conditions to produce disease-free plants of standard size, were sprayed at the rate of 4 ml. per pot with treatment chemical. The chemical PHDH was used alone at various rates and also in conjunction with the surface active agents "Cirrasol" ALN—WF and "Triton" X—100. One day later the plants were inoculated with spores of the disease. The aqueous inoculation suspension included 0.05% Tween 20 ("Tween" is a Registered Trade Mark) and contained approximately 400,000 spores per millilitre. It was applied at a rate of 4 mls per pot, an amount sufficient to wet the plants.

The plants were then placed for 24 hours in a cabinet in which a temperature of 65°F was maintained at 100% relative humidity. They were then removed to a glasshouse and kept above 66°F (but below 90°F) for approximately 7 days.

They were then visually assessed for disease by counting the number of lesions on the top two inches of the profile (first leaf to develop). The results are given in the table below as the average of 3 replicates (20 plants per replicate) and are expressed as the percentage amount of disease present.

Test No. 1

Amount of PHDH (p.p.m.)	Amount of Surface Active Agent in p.p.m.					
	"Cirrasol" ALN - WF(ppm)			"Triton" X - 100(ppm)		
	0	100	250	0	100	250
25	18.78	11.15	8.43	18.78	11.90	4.67
50	19.33	-	6.90	19.33	-	3.67
Untreated control			31.00			

"Cirrasol" and "Triton" are Registered Trade Marks.

It is noteworthy that improved disease control was achieved by using PHDH in conjunction with the surface active agents.

In a further test the procedure and conditions were substantially the same as above except that the plants were two weeks old before spraying and were sown in a 5" diameter pot. Also spraying was at the higher rate of 225 litres per hectare.

Results are given in the table below in which the amount of disease is expressed as a percentage number.

Test No. 2.

Amount of PHDH in p.p.m.	Amount of "Cirrasol" ALN- WF in ppm			
	0	1000	2000	4000
0.05	6.30	3.18	0.70	0.72
1	2.13	0.90	0.31	0.2
2	1.73	0.21	0.06	0.0
Untreated control	15.10			

Again the results show the benefit in terms of improved disease control achieved by incorporating surface active agent in the aqueous solution PHDH sprayed into the plants.

EXAMPLE 22.

This Example illustrates the combating of the fungal disease *Botrytis cinerea* on tomato plants using PHDH.

Tomato plants (variety Outdoor Girl) at the 2-leaf stage and approximately 3 weeks old, were sprayed with the treatment chemical at a rate of 2 ml per plant.

The plants were inoculated with the disease 24 hours later by spraying them with an aqueous suspension of spores which contained 1% by weight of sucrose. The spore suspension contained 50,000 spores per millilitre and it was applied in sufficient amount to wet the plants (i.e. maximum retention). The plants were then placed in humidity cabinets for 48 hours at 65°F and 100% relative humidity. They were then removed and kept in a glasshouse for 3 to 4 days before assessment. Assessment was visual and gradings were accorded for different levels of disease as follows:—

Grading	Disease
0	60 to 100%
1	25 to 60%
2	5 to 25%
3	1 to 5%
4	No disease

The gradings obtained are set out in the Tables below for the various tests conducted.

Test No. 1

Amount of PHDH in ppm	Amount of Surface Active Agent - "Cirrasol" ALN-WP in ppm			
	0	50	100	500
1	0.6	1.5	1.6	0.3
2.5	3.2	3.2	2.3	2.2
5	3.2	3.3	2.9	3.1
10	3.8	3.4	3.3	2.4

The advantages of incorporating surface active agent in the aqueous PHDH Solution are less clearly marked here, and indeed at the higher rates of PHDH it may be disadvantageous to add it.

Test No. 2

Amount of PHDH in ppm	Amount of Surface Active Agent - "Cirrasol" ALN-WP in ppm			
	0	50	100	500
10	2.6	2.4	2.2	1.4
25	2.8	2.9	2.2	1.7
50	2.7	2.9	3.0	2.1

The comments for the Test No. 1 results are re-inforced by the above results.

Test No. 3.

Amount of PHDH in ppm	Amount of Surface Active Agent - "Cirrasol" ALN-WF in ppm			
	0	50	100	500
1.0	2.5	2.6	3.0	2.8
0.5	0.3	1.6	2.6	2.6

At low rates of PHDH it here appeared advantageous to add a surface active agent.

In the results set out in the Table below for Test No. 4, the aqueous solutions all contained 1500 ppm of "Natrosol" 0—50.

Test No. 4.

Amount of PHDH in ppm	Amount of Surface Active Agent - "Cirrasol" ALN-WF in ppm			
	0	50	100	500
1.0	1.4	2.6	2.5	2.6
0.5	1.0	2.5	2.8	3.0

(a grading of 3.0)

Excellent control of the disease was obtained for the combination of 0.5 of PHDH, 500 ppm of the surface active agent and 1500 ppm of "Natrosol" 0—50.

EXAMPLE 23.

This Example illustrates the combating of the disease *Erysiphe graminis tritici* (wheat powdery mildew) using PHDH.

The Test procedure for both the Tests conducted were similar to those described for Test Nos. 1 and 2 of Example No. 21 except that after spraying there was a delay of 24 hours after treatment with the chemical before they were inoculated with the disease. Inoculation was effected by shaking infected plants over the test plants to transfer spores from the infected plants to the test ones. The results of the tests are set out in the tables below. In the first test the plants were grown in 3 inch diameter pots, each pot being sprayed with 4 ml of test chemical solution. In the second test the plants were grown in 5 inch diameter pots and sprayed at the rate of 225 litres per hectare. The figures given in the tables represent the percentage number of diseased plants.

Test No. 1

Amount of PHDH in ppm	Amount of Surface Active Agent "Cirrasol" ALN-WF in ppm				
	0	50	100	200	400
5	36.08	25.83	25.42	24.17	18.75
10	30.33	30.58	28.08	25.08	19.92
25	31.08	25.67	25.83	24.50	18.88
50	37.58	28.33	21.55	17.85	20.83
100	31.33	28.58	15.58	16.92	14.83
250	28.50	16.30	9.85	10.42	4.42
0					
Untreated control	27.72				

Test No. 2

Amount of PHDH in ppm	Amount of Surface Active Agent - "Cirrasol" ALN-WF in ppm			
	0	1000	2000	4000
0.5 kg/L	27.60	18.60	17.08	14.98
1 kg/L	21.48	15.95	14.48	12.58
0				
Untreated control	31.92			

EXAMPLE 24.

This Example illustrates the combating of foliage diseases on strawberry plants, vines and potato plants growing in the fi ld. The test procedure for the different plants and the diseases are set out below:—

Strawberries — *Botrytis cinerea* (grey mould)

Strawberry plants (2 years old — variety Cambridge Favourite) were sprayed to run off at 3 different rates during the flowering period on three occasions in May/June with high volume sprays containing test chemical. Assessments of the disease levels were visually carried out by harvesting the ripe fruit and recording the respective numbers of diseased and clean fruit.

The percentage number of diseased fruit is given in the table of results below:—

Treatment Chemical	Rate in ppm	Percentage No. of diseased fruit
PHDH	500	27.8
PHDH	1000	24.6
PHDH	2000	25.5
Untreated control		34.2

A significant degree of control of the disease was achieved.

Vines — *Uncinula necator* — Powdery mildew

Vines — *Plasmopora viticola* — Downy mildew

Vines (well established) were high volume sprayed to run-off four times with test chemical at 200 ppm at approximately 14-day intervals. Disease levels were visually assessed at the time of the third spray and again 3 weeks after the final spray and a grading accorded on the scale:—

0 = No disease
1 = Very slight infection
2 = Slight infection
3 = Slight-Moderate infection
4 = Moderate infection
5 = Moderate — Severe infection
6 = Severe infection

Results are given in the table below:—

Vine Powdery Mildew (*Uncinula necator*)

Chemical tested	Rate of application in ppm	First Assessment		Second Assessment
		Old leaves	New leaves	All leaves
PHDH	2000	0.40	0.00	1.00
Untreated control	-	5.20	2.20	4.00

Vine Downy Mildew (*Plasmopara viticola*)

Chemical tested	Rate of application in ppm	First Assessment		Second Assessment
		Old leaves	New leaves	All leaves
PHDH	2000	3.80	3.80	2.80
Untreated control	-	4.20	4.60	4.40

Potato Plants — *Phytopthora infestans* — Late Blight

Potato plants variety King Edward were high volume sprayed five times at 14-day intervals during the growing season with test chemical. An assessment of the disease level was carried out after the third spray.

Disease Grading Scales of 0 to 6 were accorded on the basis of a count of lesions in which 0 represented no lesions and 6 severe lesions.

Results are shown in the Table below:—

Potato Blight — (*Phytopthora infestans*)

Chemical Treatment	Rate of Application	Degree of blight infection
PHDH	2000	2.25
Captafol	1500	2.25
Untreated control	-	3.50

EXAMPLE 25.

This Example illustrates the combating of the fungal disease of Blackcurrants powdery mildew (*Sphaerotheca mors-uvae*) in glasshouse tests. The test procedure was as follows:—

Two-year old field blackcurrant bushes (variety Baldwin) pruned back in the autumn and planted in 10 inch pots were first high-volume sprayed to run-off with test chemical and inoculated 3 days later by blowing spores on to them from diseased bushes placed alongside them in the glasshouse. Two further sprays were applied at 14-day intervals after the first spray. Two visual assessments after the first and second sprays, respectively were made, the percentage number of leaves infected being counted and recorded. Results are set out in the Table below:—

Chemical Treatment	Rat of Application in ppm	Percentage Number of leaves with Mildew-infected top surfaces.	
		First Assessment	Second Assessment
PHDH	1000	31.8	10.9
Untreated control	-	45.1	30.1

EXAMPLE 26.

PHDH was tested against general foliage-borne bacterial plant diseases in the glasshouse. The anti-bacterial screening method employs a mist propagator to aid infection of treated plants by providing conditions of high humidity. PHDH proved to have some activity as an antibacterial spray under these conditions in spite of its high solubility in water.

Different experimental formulations were tested. The tests were carried out on fireblight of pears, rice blight and tomato spot.

Pear, tomato and rice seedlings were sprayed and root drenched with an aqueous solution containing 200 ppm of the test chemical. After 48 hours they were inoculated with the appropriate disease organism; *Erwinia amylovora* (fire blight) on pears, *Pseudomonas tomato* (tomato spot) in tomatoes and *Xanthomonas oryzae* (rice blight) on rice. Inoculations were accompanied by wounding the plants which is necessary for bacterial infection to take place. Immediately afterwards the plants were placed under the mist propagator. Agrimycin (17% streptomycin sulphate) at 2000 ppm and 1000 ppm was applied as a standard treatment and water as a control. After eight days, the symptoms were assessed on a 0—4 scale as shown below:—

Grade	Percentage Amount of disease
0	61 — 100%
1	26 — 60%
2	6 — 25%
3	Up to 5%
4	Disease free plants

One formulation, with a wax base, gave promising results against rice blight at the low rate of 200 ppm. Activity was also displayed against the other two diseases.

Chemical Treatment	Rate of Application in ppm	Disease Grade		
		X. oryzae	E.amylovora	Ps.tomato
PHDH Wax formulation	200	4	1	2
Streptomycin sulphate	2000	1 (Phyto)	1	4
Streptomycin sulphate	1000	4	4	4
Control		0	0	0

EXAMPLE 27.

Compositions containing polymeric hexamethylene diguanide were made up and tested against soil-borne fungal diseases. The procedure used in these tests,

and the results obtained in ach f them are shown hereinafter. The compound tested, and results, are listed in the Table below.

Test against *Pythium ultimum* — Procedure

Approximately one gram portions of culture of *Pythium ultimum* maintain d on 2% malt agar test tube slopes at 20°C are transferred to about 400 grams of sterilized soil containing to about 400 grams of sterilized soil containing 5% maize meal in a 300 ml. bottle. After 10 to 14 days the inoculated soil is mixed with sterile John Innes seed compost at a rate of 800 grams of soil culture to 32 litres of compost.

The mixture is moistened and covered and after three days is used as follows. Approximately 100 grams of the mixture is placed into a fibre pot and 10 pea seeds coated 2 days beforehand with chemical under test (a powdered dressing containing 25% by weight of the chemical was used) at the rate of 500 ppm. are sprinkled on the surface of the soil. Another 100 grams of the mixed soil is then placed on top of the seeds and the pot is kept in the greenhouse at between 16°C and 22°C. A first count of emergent seedlings is made after 10 days and another week is allowed to lapse before a second visual assessment takes place by pulling the seedlings up and inspecting their roots. Six replicates are conducted and observations are made of the number of healthy seedlings and the number of unhealthy seedlings. The number of ungerminated seeds is less than the number of emergent seedlings. Controls wherein untreated seed is used, and also standards wherein seed treated with thiram are used, are simultaneously carried out. Thiram is bis (dimethylthiocarbamoyl) disulphide. Calculations are then made whereby a grading is obtained for disease control.

Test against *Fusarium culmorum* — Procedure

John Innes seedling compost is admixed with a culture of *Fusarium culmorum* grown on an admixture of soil and cornmeal and the entire mixture then wrapped in brown paper and incubated in the glasshouse for 48 hours. The incubated soil is placed in pots; then seeds (twenty per pot) treated with a 25% seed dressing formulation containing the chemical under test in concentration 1000 parts per million are sown in pots. Seeds treated with "Agrosan" (Trade Mark) mercury seed dressing are used as a standard. Counts of the seedlings emergent 10 days after sowing are taken and the results converted to a percentage of the seeds sown. Disease assessments are made 16 days after sowing.

Test against *Rhizoctonia solani* — Procedure

An inoculum of *Rhizoctonia solani* is added to a partially sterilized loam soil, to provide the latter with a 1% w/w content of the inoculum. The loam soil is then allowed to stand for one week so as to be completely colonised by the disease.

The test compound, as a 25% powder seed dressing formulation, is then admixed with the loam soil at a rate of 100 parts per million parts of soil (by weight). After standing for four days to allow the chemical to take effect plastic pots are half-filled with untreated partially sterilized, loam soil and cotton seeds sown on the surface thereof, whereafter the pots are topped up with the treated loam soil.

A control experiment is conducted with PCNB (pentachloronitrobenzene). The pots are then inspected and assessed 13 days later for disease.

The results of the three foregoing tests are set out in the Table below, expressed as gradings as follows:—

Grading	Significance of grading
0	No activity or up to 20% of the disease control given by standard.
1	20—75% of the disease control given by standard.
2	75—99% of the disease control given by standard.
3	Degree of control equal to, or better than standard.

TABLE

Compound No.	Disease		
	Pythium ultimum	*Fusarium culmorum*	*Rhizoctonia solani*
1	0	3	0

EXAMPLE 28.

This Example illustrates the activity of polymeric hexamethylene diguanide hydrochloride against the disease *Fusarium nivale* on rye. The test procedure is carried out on 70% — infected Arsten's winter rye stock.

The infected seed is dressed with the test compound as a 25% seed dressing at a rate of 1000 ppm/weight/weight seed. Four replicates each of 20 seeds are planted 1 inch deep in 2½ inches diameter plastic pot using John Innes Seed Compost and placed in a glasshouse at 12°C for four weeks. The seeds emerging are counted and the plants are then assessed for disease symptoms which are yellowing of the leaves and browning of the stems; the plants are often stunted.

The percentage total seedling emergence, and percentage of emerged seedlings which show no disease symptoms are determined. These are expressed in comparison with the standard treatments, benomyl at 100 ppm and "Agrosan" at 20 ppm.

Test Chemical	Rate of application in ppm	Seedling Emergence (percent)	Healthy Plants (percent)
Polymeric hexamethylene diguanide hydrochloride	1000	82	18
Benomyl (50% chemical)	1000	83	15
"Agrosan" (1% mercury)	20	98	16
Untreated control	-	82	6

EXAMPLE 29.

This Example illustrates the activity of polymeric hexamethylene diguanide hydrochloride against *Septoria nodorum* (glume blotch) of wheat.

The test procedure is carried out on a 60% infected stock of Champlein wheat. The procedure followed is otherwise identical to that of Example 28.

Assessment of the disease is made by counting the number of seedlings emerged and expressing this as a percentage. These data are expressed in comparison with the standard treatments, benomyl at 1000 ppm and Agrosan at 20 ppm.

Compound	Rate ppm.	Seedling Emergence (percent)
Polymeric hexamethylene diguanide hydrochloride	1000	60
Benomyl (50% Chemical)	1000	45
"Agrosan" (1% mercury)	20	45
Untreated control	-	38

EXAMPLE 30.

This Example illustrates the use of PHDH as a seed dressing on french beans to combat haloblight *Pseudomonas phaseolicola*. French bean seed was soaked in a suspension containing 10^9 c lls per ml. of *Pseudomonas phaseolicola* for two hours, then dried for 24 hours and dressed with a 25% Dispersible Powder formulation containing PHDH at 1000 ppm on a weight/weight basis. The treated seed was bore-milled for 30 minutes and then sown in 3 inch pots in John Innes No. 1 Compost. There were five seeds planted in each pot and 5 replicate pots. French Bean seed, dressed with agrimycin (17% streptomycin sulphate) at 1000 ppm was used as a standard and untreated infected seed used as a control. The plants were scored for disease on a 0—3 scale, where;

0 = severe disease
1 = moderate disease
2 = slight disease
3 = No disease.

Treatment	Mean disease grade
PHDH - 1000 ppm	2.22
Agrimycin - 1000 ppm	2.35
Untreated seed	1.82

EXAMPLE 31.

This Example illustrates the activity of PHDH in an in *vitro test* against the virus organism Tobacco Mosaic Virus.

Aqueous solutions of PHDH at 2 g/litre and 0.2 g/litre were prepared. These solutions were mixed with equal volumes of tobacco mosaic virus inoculum so that the final solution contained 1 g/litre and 0.1 g/litre respectively of PHDH.

The combined chemical and virus solution was used to inoculate a half leaf of *Nicotiana glutinosa* and the other half of the leaf was inoculated with the virus solution to which an equal volume of water had been added. Infectivity between these two were compared. The results of the test are tabulated below:

Infectivity on half leaf of *Nicotiana glutinosa*

Chemical Treatment and rate of Application in ppm.	Average Number of lesions per half leaf	Percentage degree of virus control
1000 ppm PHDH	3.2	96.8
1000 ppm PHDH	5.0	95.0
Control (water)	100.0	0

EXAMPLE 32.

This Example illustrates the use of PHDH to extend the vase life of cut flowers.

Several experiments were conducted using different chemical treatments and different varieties of flowers. In each of these freshly cut flowers were handled in the same way, the treatment being as follows:

Approximately one inch of stem was cut out from the base of the stalk of each bloom. The flowers were placed individually into 100 ml. capacity measuring cylinders each containing 100 ml of test solution. Cotton wool was loosely placed around the neck of each cylinder to reduce evaporation. In all test solutions

deionised water was used instead of tap water and there were 6 replicate cylinders per treatment.

The criterion used to determine the vase life of the blo ms varied depending on the fl wer type on test. Control carnations curled upwards becoming 'sleepy' and finally shrivelled, wh reas the treated blooms rarely became 'sleepy' but eventually showed signs of petal scorch. Most other species were assessed when shrivelling or scorch first appeared, but roses often suffered from a condition known as 'bent neck' early on.

Test No. 1

Effect of various rates of PHDH and Sucrose on Carnations (Variety White Sim)

Chemical treatment	Vase life (days)	Percentage increase in vase life.
Water (Untreated control)	5.0	
2% sucrose	6.1	22
4% sucrose	6.3	26
4% sucrose + PHDH -10 ppm	8.0	60
4% sucrose + PHDH-100 ppm	8.8	76
4% sucrose + PHDH-200 ppm	12.0	140

High sucrose rates are known to be partially effective on carnations. However, the addition of PHDH increases 'shelf life' still further.

Test No. 2.

Comparative Effects between PHDH and Standard Compounds on Carnations — variety White Sim

	Vase life (days)	% Increase
Water	4.2	
PHDH 100 ppm + sucrose 4%	13.2	214
8-hydroxyquinoline 100 ppm + sucrose 4%	13.0	209
PHDH 100 ppm + sucrose 4% + Iso-ascorbic acid 100 ppm	15.2	261
8-hydroxyquinoline 100 ppm + sucrose 4% + iso-ascorbic acid 100 ppm	11.6	176

Tr atment	Vase life (days)	% Increas
Water (untreated control)	5.0	
PHDH 100 ppm + sucrose 4% + iso-ascorbic acid 100 ppm	10.6	112
Silver nitrate 100 ppm + sucrose 4% + iso-ascorbic acid	11.0	120

Iso-ascorbic acid was added in some cases as an anti-oxidant to further extend shelf life, although the results from the additions were variable. PHDH compared favourably with the known treatments.

The addition of growth regulators, in particular gibberellic acid, to a mixture of PHDH and sucrose, was found to increase the vase life beyond that obtained with the two-comparant mixture of PHDH and Sucrose.

Test No. 3.

Effect of PHDH on cut flowers other than Carnations. Sweet peas

Treatment	Vase life (days)	Percentage increase
Water (untreated control)	4.8	
PHDH 100 ppm + 4% sucrose	7.4	54

Stocks

Treatment	Vase life (days)	Percentage increase
Water	6.4	
PHDH 100 ppm + 4% sucrose	10.4	63

Roses — variety Spanish Sun

Treatment	Vase life (days)	Percentage increase
Water	4.8	
PHDH 100 ppm + 2% sucrose	6.4	33

The above results illustrate the prolongation of the vase life by PHDH of a variety of flower types.

EXAMPLE 33.

This Example illustrates a dusting powder which may be applied directly to plants or other surfaces and it comprises 3% by weight of polymeric hexamethylene diguanide hydrochloride (PHDH) mixed with 97% by weight of china clay.

EXAMPLE 34.

This Example illustrates an oil-in-water emulsion containing PHDH.

25 parts by weight of PHDH are dissolved together with 2.5 parts "Lissapol" NX in 45 parts of water. To this solution was added a mixture of 25 parts by weight mineral oil and 2.5 parts of "Lubrol" MOA with stirring, to give a creamy emulsion.

The emulsion is usually further diluted with water for use as a fungicidal spray.

EXAMPLE 35.

10 Parts by weight of PHDH, 10 parts of an ethylene oxide-nonylphenol condensate ("Lissapol" NX; "Lissapol" is a Trade Mark) and 80 parts by weight of dimethyl formamide were thoroughly mixed. There was thus obtained a concentrate which, on mixing with water, gave a solution suitable for application as a spray in the control of fungal and bacterial diseases.

EXAMPLE 36.

The ingredients listed below were ground together in the proportions stated to produce a powdered mixture readily dispersible in liquids.

	% wt.
PHDH	25
"Supronic" E 800	5
Spestone (China Clay)	70
	100%

EXAMPLE 37.

A composition suitable for use as a seed dressing was prepared by mixing all three of the ingredients set out below in the proportions stated.

	% wt.
PHDH	25
Mineral Oil	2
China Clay	73
	100%

EXAMPLE 38.

A granular composition was prepared by dissolving the active ingredient in a solvent, spraying the solution obtained onto the granules of pumice and allowing the solvent to evaporate.

	% wt.
PHDH	5
Pumice Granules	95
	100%

EXAMPLE 39.

This Example illustrates the preparation of a number of differently formulated aqueous sprays variously containing additives to enhance their persistence and rainfastness. For use as anti-fungal or anti-bacterial sprays these are normally diluted with water.

(i) 20 parts PHDH were dissolved in 70 parts water. To this was added a mixture of 2.8 parts Triton B 1956 (Trade Mark — modified phthalic glyceryl alkyd resin) 3.6 parts Lissapol NXP (Trade name — nonyl phenol/9 ethylene oxides) and 3.6 parts "Lubrol"MOA (Trade name — condensate of cetyl/oleyl with 2 moles of ethylene oxide), with stirring to give a cloudy solution/emulsion.

(ii) 20 parts PHDH were dissolved in 70 parts water and 10 parts "Natrosol" 250L (Registered Trade Mark — hydroxyethyl c llulose) were stirred in rapidly, with warming to give a clear, viscous solution.

(iii) 10 parts PHDH were dissolved in 40 parts water and 50 parts "Vinamul" 9900 (Registered Trade Mark — 50% polyvinyl acetate latex) were stirred in to give a milky emulsion.

(iv) 20 parts PHDH were dissolved in 70 parts water, and 10 parts PVP/VA I 535 stirred in (Trade Name — 50% polyvinyl pyrrolidone/vinyl acetate copolymer in isopropanol), to give a clear, slightly viscous, solution.

(v) 10 parts PHDH were dissolved in 40 parts water, and 50 parts "Vapor-Gard" (Trade Name — pine resin emulsion) stirred in, to give a creamy emulsion.

EXAMPLE 40.

This example illustrates a formulation containing a water-insoluble salt of PHDH for use as an anti-fungal spray.

20 parts of PHDH copper complex were mixed and dispersed into a solution of 2 parts "Cirrasol"ALN WF (Registered Trade Mark — condensate of oleyl/cetyl alcohol and 17 ethylene oxides) in 78 parts water, forming a concentrated aqueous dispersion.

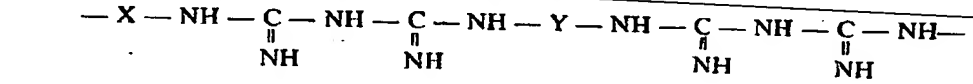
The concentrate is usually further diluted into water for use as a fungicidal spray.

The following constitutes an explanation of the compositions or substances represented by the various Registered Trade Marks and Trade Names referred to in the foregoing examples.

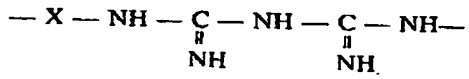
"LUBROL" L	is a condensate of 1 mole of nonly phenol with 13 molar proportions of ethylene oxide.
"LISSAPOL" NX	is a condensate of 1 mole of nonly phenol with 8 moles of ethylene oxide.
"SUPRONIC" E800	is a polyoxypropylene/ polyoxyethylene condensate.
"LUBROL"MOA	is a condensate of cetyl/oleyl alcohol with 2 moles of ethylene oxide.

WHAT WE CLAIM IS:—

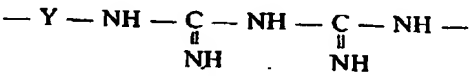
1. A method for combating fungi, bacteria and viruses which infest growing crops and the harvested produce obtained therefrom, which comprises treating the crops, or harvested produce, with a composition comprising, as an active ingredient, a polymeric biguanide or a salt thereof, which in its free base form has a recurring polymer unit represented by the formula:—



wherein X and Y, which may be the same or different, represent bridging groups $-(CH_2)_n-$ and $-(CH_2)_m-$ respectively, n and m having values from 3 to 12, or X and Y represent other bridging groups in which, taken together, the total number of carbon atoms directly interposed (as hereinbefore defined) between the pairs of nitrogen atoms linked by X and Y is from 10 to 16, and wherein the polymeric biguanide comprises a mixture of polymers in which the individual polymer chains are of different lengths, the number of individual polymer units:

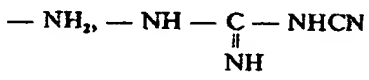


and



taken together in any polymer chain being from 3 to 80, and wherein the groups

terminating the polymer chains, which groups may be the sam r different, are selected from



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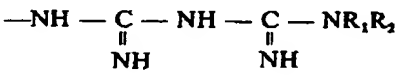
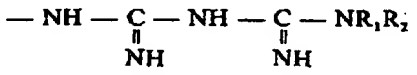


wherein R_1 is hydrogen or a substituted or unsubstituted aliphatic, cycloaliphatic, araliphatic or aromatic hydrocarbon radical containing from 1 to 18 carbon atoms and R_2 is a substituted or unsubstituted aliphatic, cycloaliphatic, araliphatic or aromatic hydrocarbon radical containing from 1 to 18 carbon atoms.

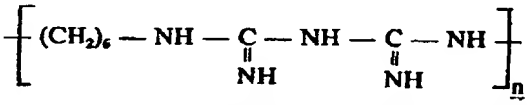
2. A process according to claim 1 wherein the bridging groups X and Y consist of polymethylene chains, which may be interrupted by hetero atoms or include saturated or unsaturated cyclic nuclei, and the groups terminating the chains are $-NH_2$ groups.

3. A process according to claim 1 or claim 2 wherein the polymeric biguanide is partially or fully terminated by a group



wherein R_1 is hydrogen and R_2 is phenyl, benzyl, cyclohexyl, 4-chloro-phenyl, 4-aminophenyl or cetyl.

4. A process according to claim 2 wherein the polymeric biguanide is poly (hexamethylene biguanide), or an acid salt thereof, represented by the formula:



wherein n has a value from 6 to 10, the average molecular weight of the polymer mixture being from 1100 to 1800.

5. A process according to any of the preceding claims and wherein the composition used comprises a surface active (wetting) agent.

6. A process according to any of the preceding claims wherein the composition used in an aqueous solution of the hydrochloride salt of the polymeric substance containing a surface active (wetting) agent.

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Agent the the Applicants.

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